"BIOLOGICAL CONTROL OF METHANE IN COAL MINES COAL STROAGE SILOS AND SHIP HOLDS PHASE II"

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GEMINI BIOCHEMICAL RESEARCH LTD.

BIOLOGICAL CONTROL OF METHANE IN COAL MINES
COAL STORAGE SILOS AND SHIP HOLDS
PHASE II

A REPORT BY

GEMINI BIOCHEMICAL RESEARCH LTD.

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SUMMARY

Water and coal samples were obtained from five Western Canadian Mines and from two Cape Breton Mines. Chemical analyses of the water samples and coal leachates from the Western Canadian mines showed that conditions in these mines were appropiate for the growth of methane oxidizing bacteria. In particular, the pH of the mine waters were in the correct range (pH 7.0 - 8.5) and there did not appear to be any toxic compounds present in the water. Conditions in the Cape Breton mines were found to be inappropriate for methane oxidizing bacteria since the waters in these mines had a much lower pH due to the activities of sulphur oxidizing bacteria on the pyrite found in association with the coal in these mines.

Methane oxidizing bacteria were found to be present in all water samples examined from the Western Canadian mines.

One type of organism was grown in large quantities and its growth requirements were partially characterized. The organisms grew in a simple inorganic (Bushnell Haas) medium when incubated in a methane/air (20/80; v/v) mixture. The optimal pH (8.0) was very close to the average pH levels found in the Western Canadian mine waters.

The organisms were equally active when the distilled water used in the liquid medium was replaced with actual mine water. This showed that there was no inhibitory compounds present in the mine water.

The organisms retained 60% of their initial activity after a period of 100 days in contact with a Balmer North coal sample. However, they rapidly lost their activity when grown in contact with a high sulphur Cape Breton coal.

When the methane oxidizing bacteria were added to a freshly crushed Western Canadian coal the organisms oxidized the methane as it desorbed from the coal. No methane was detectable after seven days whereas controls with no bacteria had levels of 7% methane.

Experiments with a dynamic test rig showed that the organisms were able to reduce the levels of methane in air flowing through a coal bed by as much as 75.2%.

RESUME

Les échantillons d'eau et du charbon provenaient de cinq mines de l'Ouest canadien et de deux mines du Cap Breton. L'analyse chimique des échantillons d'eau et de lessivage du charbon en provenance des mines de l'Ouest canadien a démontré que les conditions dans ces mines étaient compatibles avec la culture de bactéries d'oxydation du méthane. En particulier, le pH de l'eau des mines se trouvait dans la bonne fourchette (pH 7.0-8.5), et on ne trouva aucun agent toxique dans l'eau. Quant aux conditions pour les mines du Cap Breton, elles furent hostiles aux bactéries d'oxydation du méthane à cause du pH beaucoup plus bas de l'eau causé par l'activité des bactéries d'oxydation du sulfure sur la pyrite se trouvant mélangée au charbon de ces mines.

Des bactéries d'oxydation du méthane étaient présentes dans tous les échantillons en provenance des mines de l'Ouest canadien.

Un type d'organisme fut cultivé en grande quantité et les exigences de sa culture furent partiellement étudiées. Les organismes furent cultivés dans un simple milieu inorganique (Bushnell Haas) alors qu'ils furent incubés dans un mélange méthane/air(20/80; v/v). Le pH maximum (8.0) était très près du pH moyen trouvé dans les eaux des mines de l'Ouest canadien.

Les organismes restèrent aussi actifs quand l'eau distillée utilisée dans le milieu liquide fut remplacée par l'eau des mines. Ceci a démontré qu'il n'existait aucun agent inhibiteur dans l'eau des mines.

Les organismes possédaient encore 60% de leur activité initiale après une période de 100 jours passés au contact d'un échantillon de charbon de Balmer Nord. Néanmoins, ils perdent rapidement leur activité au contact du charbon à haut degré de sulfure du Cap Breton.

Quand des bactéries d'oxydation du méthane furent ajoutées à du charbon fraichement broyé de l'Ouest canadien, les organismes oxydèrent le méthane qui s'échappait du charbon. Aucune trace de méthane ne put être détectée après sept jours, alors que des tests sans bactéries arrivèrent à 7% de méthane.

Des essais dynamiques avec une installation expérimentale démontrèrent que les organismes étaient capables de réduire de 75.2% les niveaux de méthane dans l'air passant à travers un gisement de charbon.

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INTRODUCTION

Most coal mine explosions have been caused by the emission and accumulation of methane. To ensure a safer mining environment and to maintain productivity at the desired level methane control at all phases of mining is considered essential.

A review of techniques developed for dealing with methane in coal mines indicate that control by ventilation alone may not be the answer to all mining situations. Alternate methods such as methane drainage during mining operations or in advance of mining showed promising results (1,2,3).

So far, little attention has been paid on the application of biotechnology for methane control. Attempts were made in Eastern Europe (4,5) and more recently in India (6) to use methane oxidizing micro-organisms for removing methane in coal mines.

A complete review on the biological control of methane was recently completed through a CANMET contract (7).

The report summarized three main areas:

1) An overview of field tests carried out in India and Eastern Europe.
2) Occurrence and properties of methane oxidizing micro-organisms.
3) Method of application.

1) Overview of field testing

Preliminary studies performed in coal mines have indicated that biologically enhanced methane control has possible uses in coal mines. Ivanov et al. (4) treated a 30mx20m surface section of coal adjacent to a coal longwall with a fermenter grown culture of a methane oxidizing bacterium Methylococcus capsulatus. In this mine 70% of the methane came

from worked out areas. Over a 15 day period the bacterium oxidized from 6.3%of the methane emitted from the treated area on day 1, to a high of 40% by day 9. By day 15 the amount of methane oxidized had fallen to 13% of the total methane emitted by the treated area. The actual volumes of out gassed methane were not reported. Abramov (5) found that the bacterium Methylomonas methanica could over an unspecified time, oxidize all the methane in a coal seam after the organism had been injected into the seam.

Thakur and co-workers (6) in India also carried out field testing in three different mines. In one mine there was an approximate 50% decrease in emission of methane three days after a nutrient solution and methane oxidizing micro-organisms were injected into the coal seam. In a second test there was complete removal of methane two months after injection of bacteria and nutrients. In the third test the methane emission rate was reduced by approximately 66% 20 days after injection of the organisms. These authors did not add oxygen to the coal and claim to have isolated an anaerobic strain of organism. However, they did not give any details on the apparent mechanism of this reaction.

2) Occurrence and properties of methane oxidizing micro-organisms

The existence of methane oxidizing bacteria was first noted by Kaserer in 1905 (8). Sohngen in 1906 (9) was the first to isolate an organism able to grow on methane as a carbon source. Since that time a large number of methane oxidizing bacteria, many of them uncharacterized, have been isolated (10). A limited number of non-bacterial micro-organisms capable of oxidizing methane have also been described (11,12).

Methane oxidizing bacteria have been isolated from a large variety of sources, and coal mines (7). The very wide range of habitats and locations that these organisms have been isolated from suggests that these

organisms could be considered ubiquitous.

The number of yeast, fungi, and algae that have been described that oxidize methane are limited. They have been isolated from lakewater, sewage, mud and soil (7).

Reports of the isolation of methane oxidizing bacteria from coal mine environments are limited and originate from Eastern Europe. Malashenko and Romonovskaya (13) examined 500 samples from coal mines and soils in the U.S.S.R. and found that most of the samples contained methane oxidizing bacteria. Ivanov et al. (14) reported that of 76 water samples taken from 14 coal mines in the U.S.S.R.,57 or 75% of the samples contained methane oxidizing bacteria. A Polish study (15) found that all of 200 water samples taken from Polish coal mines were found to contain methane oxidizing bacteria. The isolation regime that led to this high success rate included simulating the temperature, pH, and ionic constitution of the mine waters involved in the isolation media used in the laboratory. These studies suggest that conditions suitable for supporting the growth of methane oxidizing bacteria exist in many coal mines.

Canadian studies on the isolation of methane oxidizing bacteria are limited. Mueller (16,17) has found methane oxidizing bacteria in sewage and Roth-wood (18) has found them in soils in Southern Alberta.

Thus methane oxidizing micro-organisms are found in many habitats including coal mines. The physiological properties of the organisms were found to be consistent with the properties found in most coal mines (7). In particular the following parameters were found to be favourable for their use:

a) Temperature

The temperature optima for most methane oxidizers fall in the range 15 - 40°C which is within the range found in most coal mines. For cooler temperature applications, psychrophilic organisms could be isolated and used.

b) pH

The pH optima for these organisms were found to be between 5.0 and 8.0 which is similar to the pH of most mine waters studied.

c) Pressure

Pressure effects were not considered to be a factor since a large variety of organisms were shown to be capable of being subjected to high pressures without showing adverse effects.

d) Ion effects

Because of the various ionic requirements of the methane oxidizers, organisms would be isolated from waters associated with the particular mine chosen for potential application so that they would be acclimatized to that particular ionic environment.

e) Oxygen requirements

The study concluded aerobic organisms require a large supply of oxygen and could not be used for infusion to a coal seam. However, if anaerobic organisms were available they could be used with a suitable electron acceptor e.g. nitrate or sulphate ions. Availability of oxygen should not be a problem for surface applications or for use in ventilated coal storage silos.

f) Physical characteristics

Bacteria would be the organisms of choice since their smaller size would permit better penetration to the coal mass.

3) Method of Application

There are four main methods for the application of the methane oxidizing micro-organisms:

a) Infusion

Water infusion is a procedure commonly used in coal mines to aid in the control of dust. The micro-organisms could be added to the infusion water and thus could be pumped into the coal seam. This method, however has two serious drawbacks. Firstly, the availability of oxygen would be a major problem. The use of anaerobic organisms utilizing sulphate or nitrate ions as electron acceptor could overcome this problem. The second limitation is the build up of heat which would occur if adequate oxygen supplies were available to the organisms. Less heat would be produced if anaerobic organisms were used (7).

b) Surface application

This method would appear to be the simplest to use and would certainly be the method of choice for initial field tests. The only apparent limitation to this method would be in applying enough of the organisms to ensure adequate removal rates. Heating effects and oxygen availability would not be a problem. Ionic constituents leaching from the coal may be troublesome but resistant strains could be selected.

c) Storage and transportation systems

In general, the use of the organisms in these systems would have the same limitations as those found in coal mines. The two prime limitations which would have to be overcome would be availability of oxygen and temperature.

As described previously, methane oxidizing micro-organisms require large amounts of oxygen in order to oxidize methane. Thus completely closed systems will rapidly use up the available oxygen and further oxidation would cease. Thus there must be some method to allow controlled access of oxygen for complete removal of methane. Anaerobic organisms may be the answer, especially if nitrates could be added to the coal. The use of sulphate as an oxygen source would not be recommended because of possible hydrogen sulphide gas formation.

The second limiting factor would be temperature. However, it should be possible to isolate psychrophillic (i.e. cold loving) organisms. Such organisms should be present in the sediments of large, cold water lakes and in Arctic deltas.

d) Biological gas scrubbers

In cases where conditions in the mine or storage area are very extreme (e.g. low pH, high levels of toxic compounds, low temperatures) the organisms can be incorporated in a biological scrubbing device where their environment can be controlled and conditions for maximum activity can be maintained.

In summary, biological methane control appears to be a feasible alternative to other methods for reducing the hazards associated with methane in coal mining situations.

Thus further research on this area was initiated with the following objectives:

1. To isolate methane oxidizing organisms from samples collected from operating coal mines.
2. To maximize the methane oxidizing activity under simulated mine conditions.
3. To carry out static and dynamic testing of the organisms in a laboratory model.

EXPERIMENTAL

1) Sampling

Table 1 lists the mines from which samples of water and coal were obtained. A brief description of the coal mined at these sites is given in Table 2.

Water samples (500 mls) were collected in sterile containers which were tightly sealed and were kept as cool as possible until they were returned to the laboratory. Water was collected from both standing water and from water flowing down the walls of the mines.

Coal samples were collected from coal dumps and from fresh workings. The coal (1000g) was placed in plastic bags for transport to the laboratory.

2) Isolation of methane oxidizing bacteria

Two techniques were used for the isolation of the methane oxidizing bacteria, namely, isolation on solid medium and liquid medium.

a) Isolation on solid medium

0.1ml aliquots of the water samples were spread on Bushnell Haas agar plates. The solid medium was made up as follows:

Magnesium sulphate	0.2g
Calcium chloride	0.02g
Monopotassium phosphate . .	1.0g
Dipotassium phosphate . . .	1.0g
Ammonium nitrate	1.0g
Ferric chloride	0.05g
Bacto agar	1.5g
Distilled water	1000mls

The plates were incubated in a vacuum desicator (under slightly reduced pressure to ensure proper sealing of the lid) in an atmosphere of

TABLE 1.

Mines from which Water and Coal Samples were obtained

Company	Mine	Location
Westar Mining Ltd	Balmer North	S.E. British Columbia
	Balmer South	S.E. British Columbia
Smokey River Coal	Grand Cache	W. Central Alberta
Luscar Ltd.	Cardinal River	W. Central Alberta
	Coal Valley	W. Central Alberta
Devco	Lingan	Cape Breton, N.S.
	Prince	Cape Breton, N.S.

TABLE 2.

Description of Various Coals in the Study

Mine	Rank of Coal	Sulphur Content
Balmer North	medium volatile bituminous	.31%
Balmer South	medium volatile bituminous	.31%
Grand Cache	low volatile bituminous	.43%
Cardinal River	medium volatile bituminous	.30%
Coal Valley	high volatile C bituminous	.31%
Lingan	high volatile A bituminous	1.45%
Prince	high volatile A bituminous	5.10%

methane and air (20:80; v/v). The plates were checked for growth after seven days.

b) Isolation in liquid medium

One ml aliquots of the water samples were added to five mls of sterile Bushnell Haas liquid medium (same as solid medium except that no Bacto agar was added) in 30ml syringe vials. Fifteen mls of methane were added through the syringe cap. One ml of the head space gas was removed and analyzed for methane and carbon dioxide to determine initial concentrations. The vials were incubated at 30° C. Samples of the head space gas were removed and analyzed after 5 and 12 days. A positive indication of the presence of methane oxidizing bacteria in the samples was obtained if there was a decrease in methane concentration accompanied by the appearance of carbon dioxide. The disappearance of methane by itself was not sufficient reason to conclude that methane oxidizing bacteria were present since a simple decrease in methane concentration could be explained in a number of alternate ways, for example, a leak in the system or adsorption of the methane onto the walls of the reaction vessel. Sterile controls were run for comparison.

3) Isolation of methane oxidizing bacteria from coal samples

Coal samples obtained from the Balmer North mine were crushed to a fine powder in a mortar and pestle. One gram aliquots of this crushed coal were incubated at 30° C over night on a rotatory shaker. The coal was allowed to settle and the supernatant was tested for methane oxidizing bacteria in the same manner as the water samples.

4) Production of large quantities of cells

A rapidly growing culture was obtained after three or four transfers, in liquid medium, of the organisms isolated from the Balmer South water

samples. Larger quantities of cells for experimental purposes were obtained in a simple bioreactor consisting of a 500ml Erlenmeyer flask with a side arm which contained 200ml of Bushnell Haas liquid medium. The top was sealed with a rubber stopper to which a vapor lock was attached and the side-arm was capped with a syringe cap. A mixture of methane and air (20:80; v/v) was slowly admitted to the flask at a rate of 20mls per hour through a syring needle inserted in the cap. The flow of gas was maintained at this rate by pumping water, via a peristaltic pump, at a controlled rate into a large reservoir of the gas mixture. The vapour lock prevented the admittance of air to the system. The medium was stirred with a magnetic stirring bar and the bioreactor was incubated at 30°C. An innoculum of methane oxidizing bacteria (10% of medium volume) was added and the cells were harvested by centrifugation 5-7 days later. The pelleted cells were made into a slurry by adding an equal volume of Bushnell Haas medium. This slurry was used as the innoculum in all further experiments.

5) Optimization of growth conditions

The following conditions were varied to determine optimum growth conditions:

pH of the medium
aerated versus non-aerated cultures
addition of coal mine water

6) Methane oxidation in the presence of partially degassed coal

Coal obtained from the Balmer North mine was crushed into approximately lcm pieces and was allowed to degas in contact with the air for 4-7 days before being used in experiments. The coal was degassed before use so that there would be little methane desorbing from it

during the course of the experiment which would interfere with the measurement of the oxidation of the methane added during the experiments. Two hundred grams of the degassed coal were placed in 250ml Erlenmeyer flasks with side arms and 5ml of cell slurry and 20ml Bushnell Haas medium were added. The tops of the flask were sealed with rubber membranes and the side arms were fitted with syringe caps to permit addition of methane and withdrawl of samples. Thirty mls of air were removed from the flask using a plastic syringe and 30mls of methane added. Three mls of the gas were removed and analyzed to determine the concentration of methane at time zero (this was normally between 12 and 15%). The flasks were incubated at 30°C and the oxygen, methane and carbon dioxide concentrations were determined by gas chromatography after 24 hours.

7) Methane oxidation in the presence of fresh coal

Fresh coal from Balmer North mine was crushed into 1cm lumps and 200g were placed in a 500ml Erlenmeyer flasks equipped with side-arms. After addition of 5mls of cell slurry and 20mls of Bushnell Haas medium the tops of the flasks were sealed as described above. Samples of air were removed at regular intervals and analyzed for oxygen, methane and carbon dioxide. Controls in which no cells were added were run for comparison.

8) Longevity of the methane oxidizing activity in the presence of Balmer North coal

Two flasks, prepared as described in (6) above, were filled with 200g of crushed, degassed Balmer North coal. Five mls of concentrated cell slurry and 20mls of Bushnell Haas liquid medium were added. The flasks were sealed and the gas inside was made to 12% methane. Twenty four hours later samples of the gas were withdrawn and analyzed by gas chromatography for methane and carbon dioxide. The seals were removed

and fresh air was allowed into the flasks. They were resealed and methane added to make a final concentration of 12% once more.

After a further 24 hours samples were withdrawn and analyzed for methane and carbon dioxide. This cycle of events was repeated daily for a period of 100 days. A plot of methane oxidized in the 24 hour period against age of the bacterial cells was constructed (it should be noted that over the weekends the methane oxidized in a 72 hour period was measured).

9) Dynamic test rig

A dynamic test rig was assembled using a plexiglass gas drying tower (30 X 5cms). The rig is illustrated in Plate 1 and Figure 1. The tower was filled with crushed Balmer North coal and 5mls of cell slurry and 20mls of Bushnell Haas medium were added. Air-methane mixtures were pumped into the tower via the bottom side-arm. Effluent gas was allowed to leave the system via a syringe needle inserted through a syringe cap fitted to the top side-arm. Samples for analysis were withdrawn through the syringe cap. The concentration and flow rates of the influent gas were varied.

10) Analytical techniques

Chemical analyses of the coal mine waters and coal leachates were only carried out on the Balmer North and Balmer South mines since the waters and coals from the other Western Canadian mines were expected to be very similar in composition. In addition these were working underground mines where methane is a problem.

Gases were analyzed by gas chromatography. The experimental details of this technique and other methods are outlined in Appendix A.

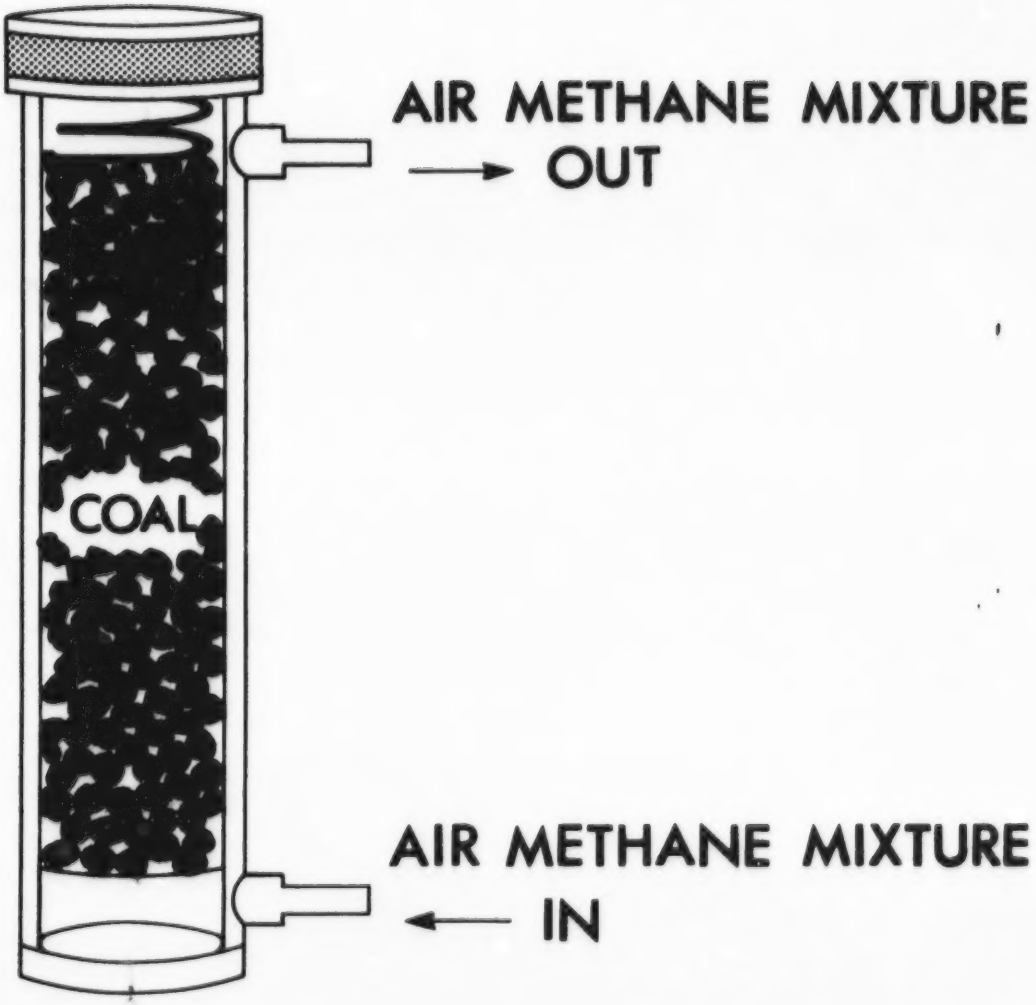


Figure 1. Dynamic Test Rig

A

B

Plate 1.

A. The dynamic test rig

B. The system used for measuring the bacterial activity in the presence of various coals.

RESULTS

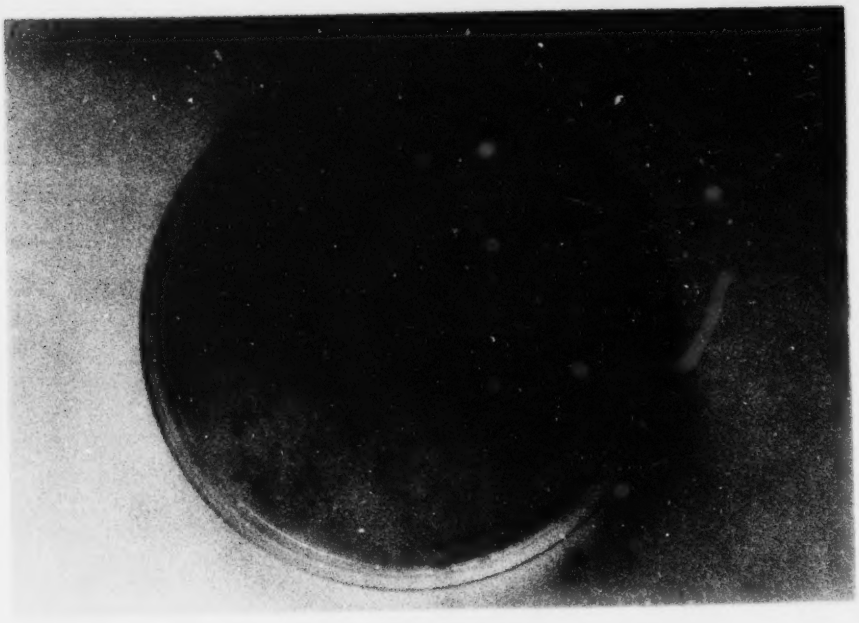
1) Isolation of methane oxidizing bacteria

Two methods were used for isolating and detecting the presence of methane oxidizing bacteria, namely, growth on solid medium and growth in liquid medium. When liquid media were used samples were considered positive when a decrease in methane concentration was accompanied by a corresponding increase in carbon dioxide. Water samples collected from Balmer South, Balmer North, Grand Cache, Cardinal River and Lingan were positive for methane oxidizing bacteria. No bacteria were observed in water samples from Prince Mine, Nova Scotia. Wet coal samples from Balmer South and Balmer North were also positive whereas dry samples were negative.

The use of solid media for the detection of methane oxidizing bacteria was found to be more sensitive but required longer incubation times. All water samples tested in this manner gave results which averaged 4000 organisms/ml. In addition to the large colonies on the plates some samples had large numbers of micro-colonies which were very hard to quantify since they tended to grow very slowly, or not at all on diluted samples. Plate two illustrates the type of colonies which grew on the solid media. They could be grouped into four main types:

large pink colonies
pink micro-colonies
small yellow colonies
small colourless colonies

The large pink colonies were picked for further study since they appeared to be the fastest growing. The colonies observed on the plates changed to salmon colour on repeated subculturing. Microscopic examination of this "culture" showed it to contain two distinct types of cells, Gram



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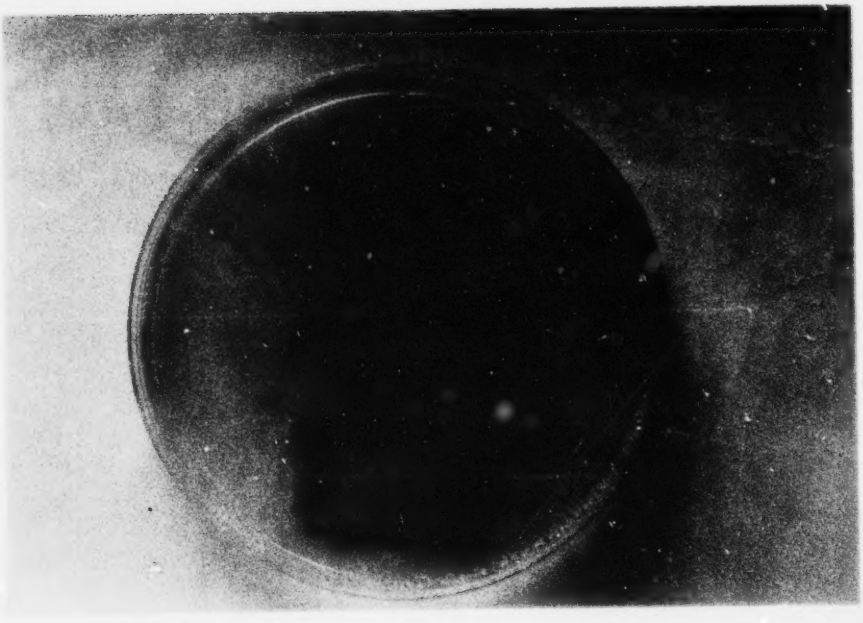
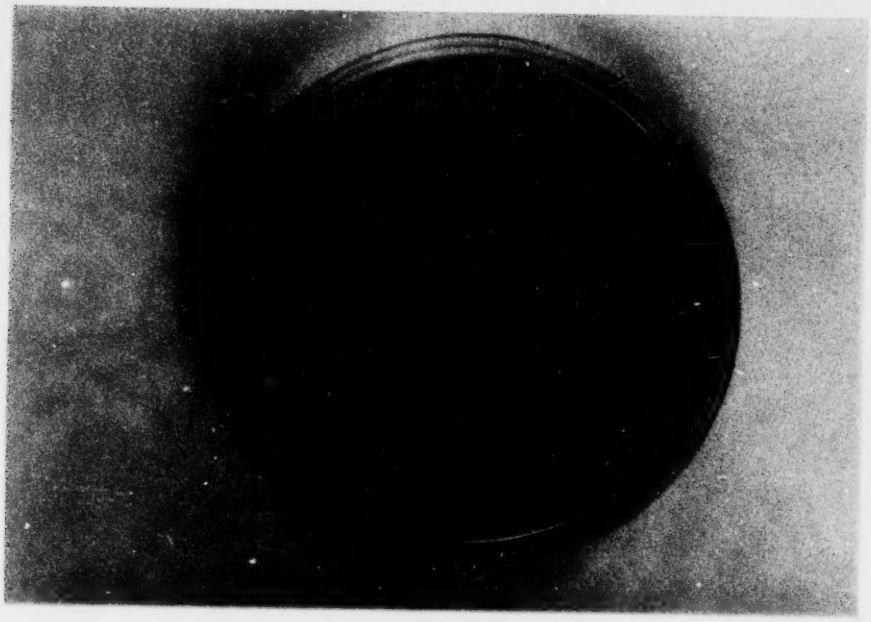


B

Plate 2

A. Methane oxidizing bacteria growing from a sample from Grand Cache

B. The yellow colonies isolated from Grand Cache.



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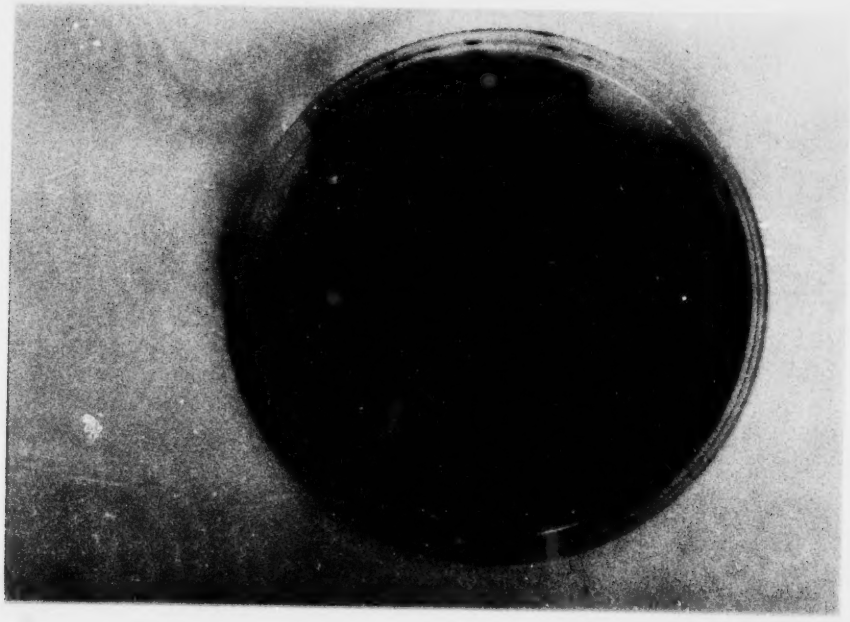


D

Plate 2. (contd)

C. Colonies isolated from Balmer South.

D. Samples for the bioreactor.

negative rods and small Gram negative cocci. No detailed classification was undertaken but the cells resembled the description of Methylomonas methano-oxidans and M. methanica.

2) Chemical analysis of mine waters

The chemical analyses of mine waters and coal leachates are shown in Tables 3 and 4. The analyses show that there does not appear to be any compound present in high enough concentration to markedly affect the activities of the methane oxidizing bacteria (19). Table 5 shows the pH of several waters tested. The majority of the samples (all from Western Canada) have pH values varying between 7.0 and 8.5 which is within the correct range for methane oxidizing bacteria (20). However, the pH of water samples associated with high sulphur coal (Prince) were much lower and were outside the range for normal methane oxidizing bacteria (5).

3) Preliminary characterization of growth conditions

The cells grew rapidly in agitated shake flasks when cultured in Bushnell Haas medium and in an atmosphere of 15-40% methane. However, growth was limited in these experiments due to depletion of methane and/or oxygen. For higher cell yields the organisms were grown in a bioreactor which permitted the continuous flow through of a methane/air mixture (20:80; v/v). Not withstanding the above, however, these small flasks were used to partially characterize the growth conditions for the organisms. Table 6 illustrates the effect of the pH of the medium on the activity of the organisms. Best results were obtained at pH 8.0. Surprisingly there appeared to be a dramatic decrease in activity when the pH was altered by even as much as 0.5 pH unit on either side of the optimal value.

To determine the effects of trace minerals (mostly elements such as Fe, Cu, Zn, Mn etc.) on the activity of the methane oxidizing bacteria

TABLE 3.

Anionic Analysis of Two Mine Water Samples[a]

Anion	Water from: Balmer North[b]	Balmer South[c]
chloride	18.0	0.6
sulphate	1.8	0.7
phosphate	< 0.1	< 0.1
nitrate	0.56	1.5

a - All concentrations in ppm.

b - Standing water which was collecting a long time.

c - Flowing water from ground drainage pipe.

TABLE 4.

Cationic Analysis of Two Mine Waters and Coal Leachates[a]

Cation	Balmer North		Balmer South	
	coal leachate[b]	water[c]	coal leachate[d]	water[e]
Ag	0.22	< 4ppb	0.02	< 4ppb
Al	25.67	0.18	16.84	0.38
As	< 25ppb	< 25ppb	< 25ppb	< 25ppb
B	4.31	0.13	0.29	0.02
Ba	6.81	12.74	9.79	6.36
Ca	2900	105.2	64.42	114.8
Cd	2.95	< 1ppb	0.09	< 1ppb
Cr	1.20	0.02	0.15	0.02
Cu	2.51	2.38	2.82	1.90
Fe	3535	1.57	98.41	0.69
K	5.55	7.99	13.84	5.71
Mg	1624	40.75	26.86	41.61
Mn	13.68	0.02	0.29	0.01
Mo	0.79	< 5ppb	0.06	< 5ppb
Na	5.95	16.38	8.50	1.13
Ni	1.66	0.24	0.29	0.14
P	27.9	0.17	1.46	0.10
Pb	5.5	< 20ppb	1.19	0.02
Se	< 20ppb	< 20ppb	< 20ppb	< 20ppb
Si	10.77	1.73	22.21	2.76
Sn	0.02	< 6ppb	< 6ppb	< 6ppb
V	2.62	0.004	0.23	< 2ppb
Zn	5.72	< 2ppb	1.00	< 2ppb

a - All concentrations in ppm unless otherwise stated.

b - Coal collected at working face.

c - Standing water which was collecting for a long time.

d - Coal collected from unworked face.

e - Flowing water from unworked face.

TABLE 5.

pH of Mine Water Samples

Mine	Type of Samples	Number of Samples	Range	Mean
Balmer North	standing and flowing water	10	7.33-8.55	8.10
Balmer South	standing and flowing water	17	7.29-8.73	8.09
Prince	standing water at bottom of working face	1	3.14	3.14
Lingan	standing water near working face	1	6.01	6.01
Cardinal River	standing water on mine roadways	4	7.77-8.78	8.30

TABLE 6.

Effect of pH on the Bacterial Activity

pH	Activity (normalized to 100 @ pH 8.0)
5.0	25.3
6.0	16.1
7.0	34.0
8.0	100.0
8.5	15.7
9.0	16.3

TABLE 7.

Effect of Mine Water on The Bacterial Activity

Bushnell Haas Medium made up in	Methane Added (mls)	Methane Oxidized in 24 hours(mls)	Percent Oxidized
distilled water	30	27.7	92.3
coal mine water	30	28.6	95.3

TABLE 8.

Mehane Oxidation Rates Under Different Experimental Conditions

Conditions	Effect	Methane Added (mls)	Methane Oxidized in 24 hours (mls)
No shaking	Low dissolved oxygen concentration; low surface area	30	5.0- 5.5
Shaking	higher dissolved oxygen concentration; higher surface area	30	8.7-18.6
Addition of coal	very high surface area easy access to oxygen	30	20.0-30.0

an experiment was conducted in which the distilled water used to make up the Bushnell Haas medium was replaced with tap water and coal mine water. As can be seen from Table 7 neither tap water nor coal mine water had any significant effect on the activity of the organisms.

4) Methane oxidation in the presence of degassed coal

The majority of the experiments measuring the bacterial methane oxidizing activity in the presence of coal was carried out on the Balmer North Samples since this is a working underground mine where methane is a problem. This coal is similar to the other western Canadian coals.

The rate of methane oxidation in the presence of degassed coal from Balmer North mine is illustrated in Figure 2 . The data show that there is a rapid oxidation of the methane under these conditions. In fact, Table 8 shows that the addition of coal to the bacterial cultures has the same effect as shaking the flasks. Thus there are no inhibitory compounds present in the coal after it has been allowed to degas for several days.

5) Methane oxidation in the presence of fresh coal

Figure 3 illustrates the effectiveness of methane oxidizing bacteria in controlling the levels of methane desorbing from fresh coal. No methane could be detected in the flasks to which the bacteria were added whereas controls with no bacteria had methane levels of 7% after 4 days.

6) Longevity of the methane oxidizing activity

The longevity of the bacterial activity is well illustrated in Figure 4. The bacterial activity remained essentially unaltered for a period of approximately 72 days, at which piont the activity began to decrease and

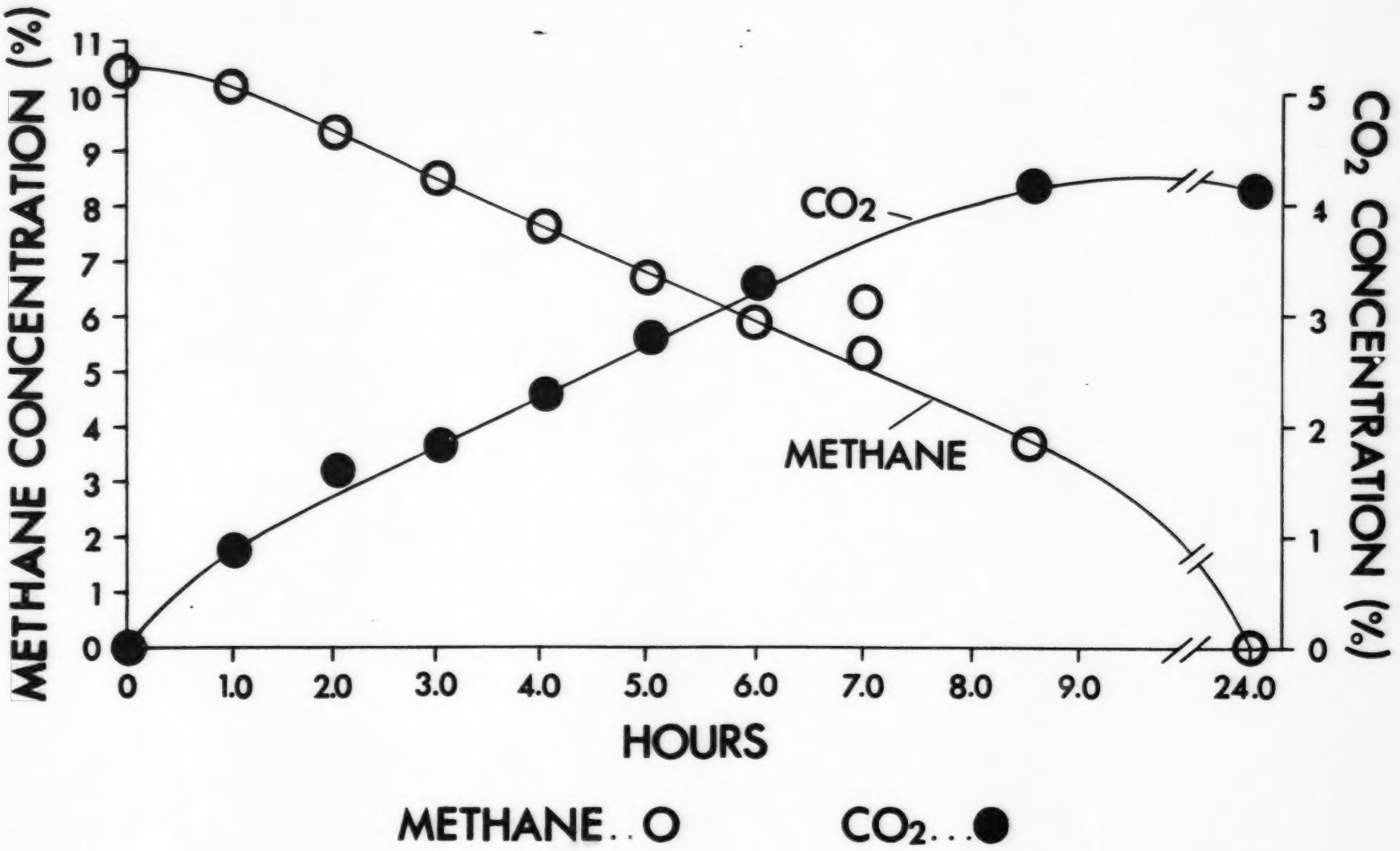


Figure 2. - Rate of disappearance of methane by methane oxidizing bacteria in coal samples. Five ml cell suspension, 200g coal and 20 ml Bushnell Haas medium per flask. Atmosphere made to 10% methane.

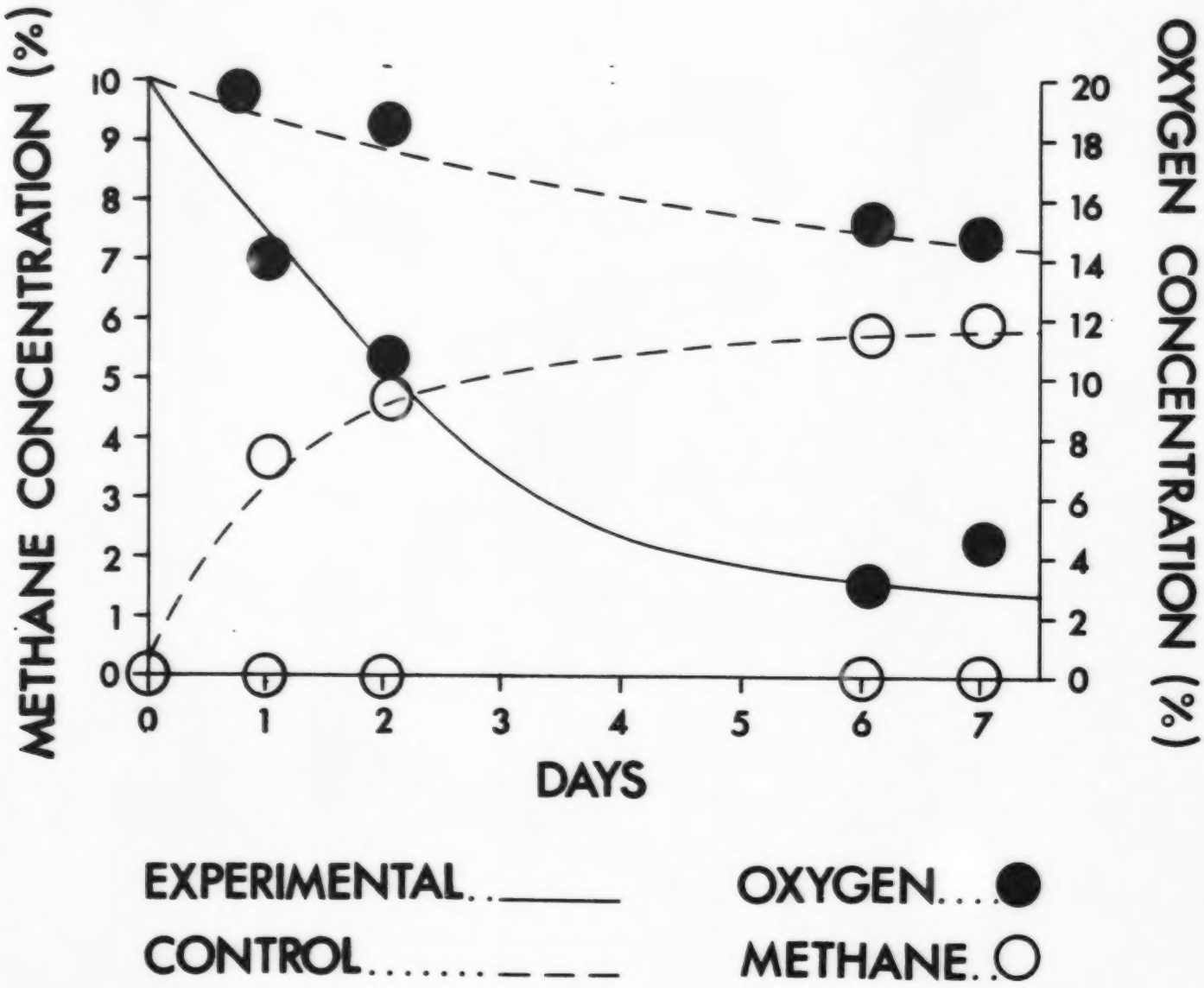


Figure 3. - Control of methane concentration in a model coal silo. Five ml cell suspension, 20 ml Bushnell Haas medium, added to 200g freshly ground coal in sealed flask.

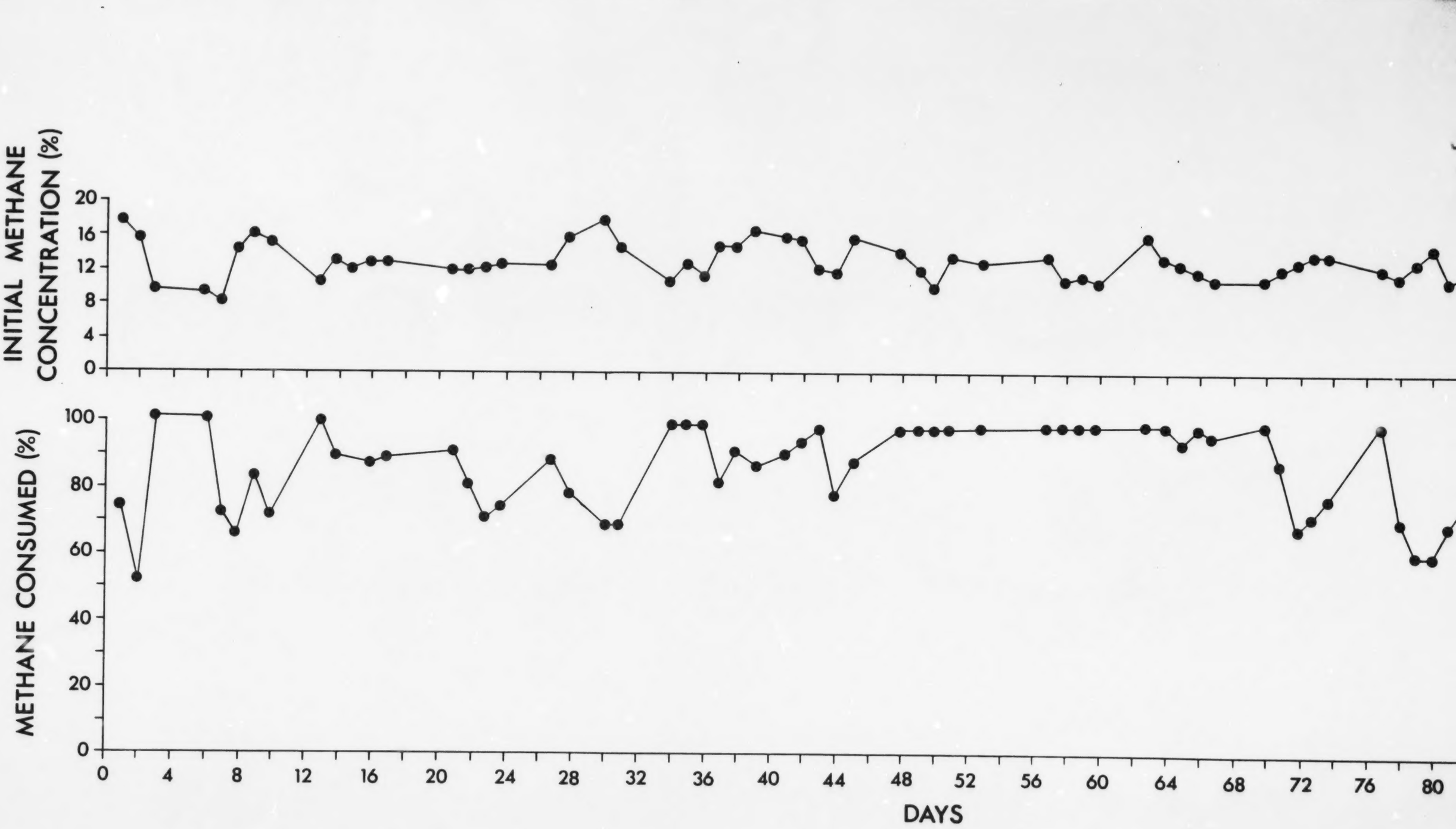
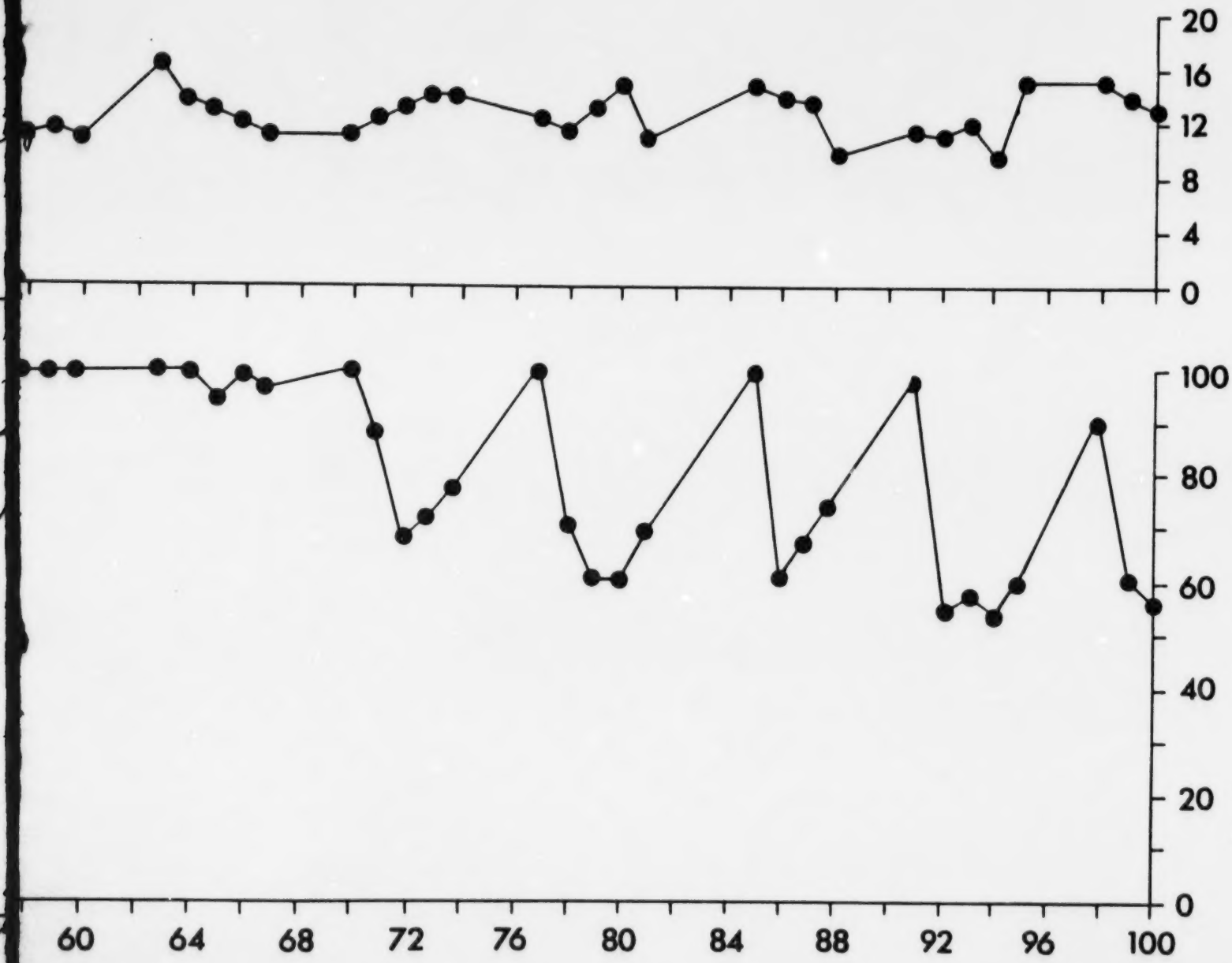


Figure 4. - Longevity of the methane oxidizing bacteria in the presence of coal. Five ml cell suspension added to 200g coal and 20ml Bushnell Haas medium. Atmosphere made to 12% methane.



coal. Five ml cell

tmosphere made to 12% methane.

after a total of 100 days, was only 60% of the inital value. During this period a total of 75 cycles of methane oxidation had been performed. Figure 4 shows the difference in the longevity of the activity when the Bushnell Haas medium is replaced with distilled water. A decrease in activity was first noticed after 26 days and activity fell to 40% of inital values after 70 days. A similar loss in activity was observed when the amount of cells added was reduced. This is shown in Figure 5.

A comparison of the longevity of the activity in the presence of five different coals is shown in Table 9. The activity in the presence of low sulphur Alberta and British Columbia coals remained high over a period of four weeks whereas the activity in the presence of a high sulphur Cape Breton Coal was rapidly lost.

7) Dynamic test rig

The results of experiments using the dynamic test rig are summarized in Table 10. These data show that the methane oxidizing bacteria are capable of oxidizing methane in gas mixtures when the gas is passed over the coal in a continuous manner. The best results were obtained at a flow rate of 1 volume/volume/day with 14.1% methane when the methane concentration was reduced by 75.3%.

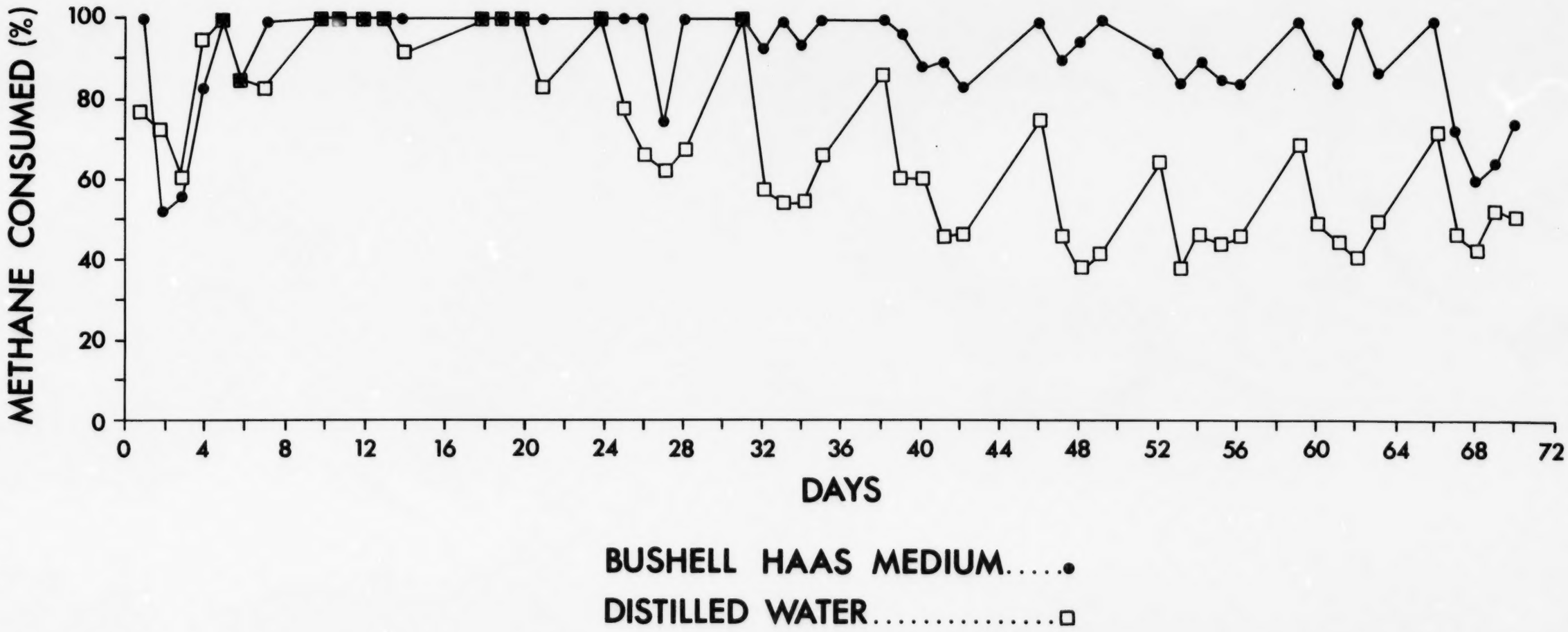


Figure 5. - Longevity of the bacterial activity when the Bushnell Haas medium used to wet the coal is replaced with water.

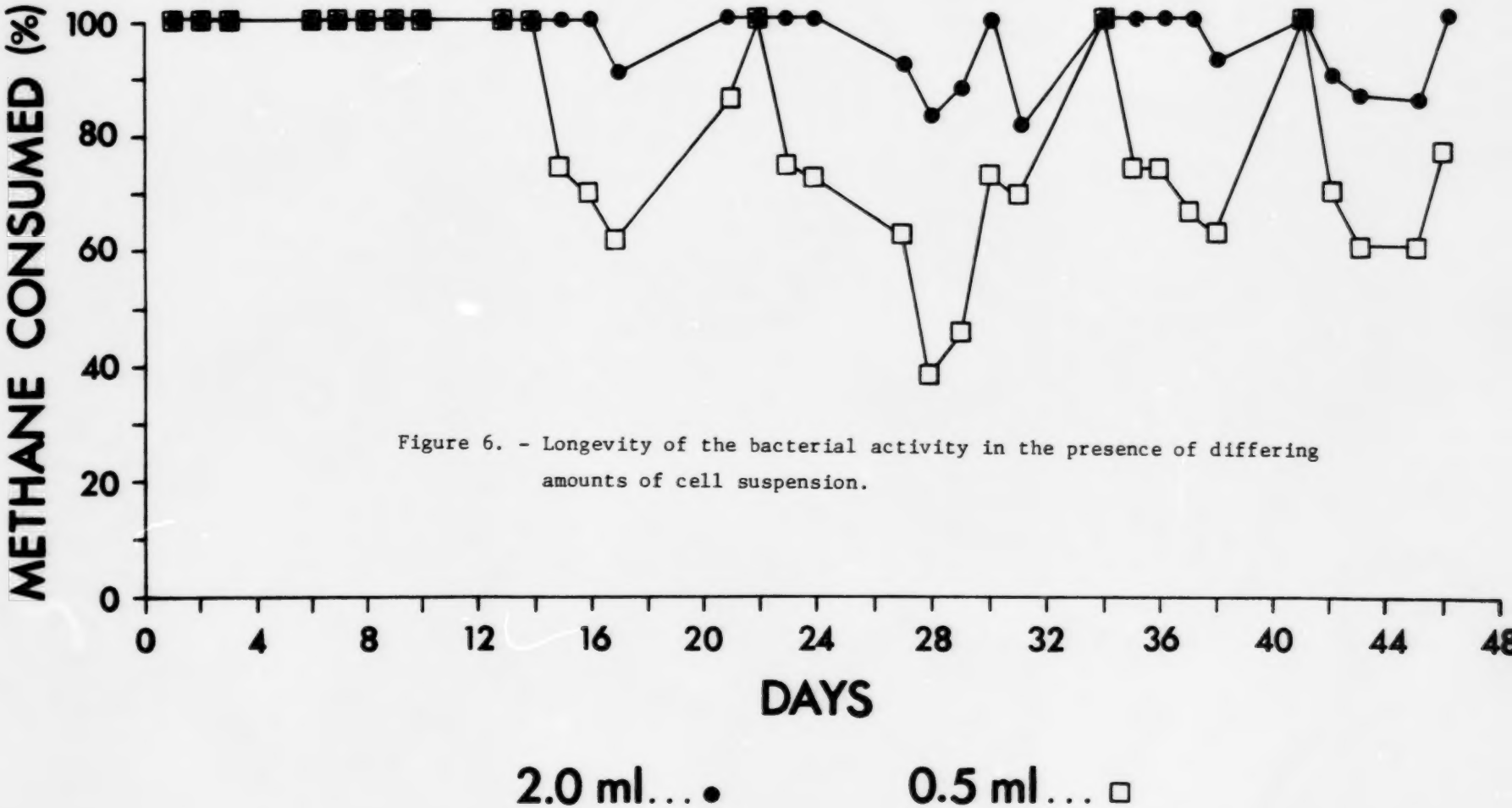


Figure 6. - Longevity of the bacterial activity in the presence of differing amounts of cell suspension.

TABLE 9.

Longevity of Bacterial Activity in Contact with Various Coals

Coal	Changes in Bacterial Activity (%) with Time.		
	5 days	12 days	16 days
Prince	21.56	53.18	27.27
Balmer North	88.09	94.63	89.01
Coal Valley	100.0	100.0	87.33
Grand Cache	100.0	100.0	100.0
Cardinal River	100.0	100.0	94.17

TABLE 10.

Summary of the Results Using the Dynamic Test Rig

Flow of Methane/Air Mixture through the Rig	Influent Methane Concentration (%)	Effluent Methane Concentration (%)
1 vol/vol/day	11.95	7.33
	6.76	2.35
	14.1	3.49
2 vol/vol/day	13.46	3.61
	6.76	2.58
3 vol/vol/day	14.06	9.28
	6.39	5.43

DISCUSSION

The results of a feasibility study on the biological control of methane in coal mines came to the conclusion that bacteria would be the most suitable organisms for this purpose (7). This choice was based on the physiological properties, the relative rates of methane oxidation and the physical properties of the organisms. Thus during this study the techniques used to isolate micro-organisms from the coal mine samples were appropriate only for bacteria and would not select for yeasts nor fungi. The majority of methane oxidizing bacteria reported in the literature (19,20,21) will grow on simple inorganic media with out the supplementation of complex organic compounds such as vitamins, amino acids and other growth factors. Thus only a simple inorganic medium, Bushnell Haas, was used in this study.

The chemical composition of the water samples obtained from the Western Canadian coal mines were quite compatible with the activity of the methane oxidizing bacteria. In particular, the pH observed (7.5-8.5) was close to the maximum range previously noted(7) and there was not a high level of any potentially toxic metal, even in the acid leached samples. This was borne out by the fact that large numbers (4000- 10,000 **per ml**) of methane oxidizing organisms were found in the majority of water samples taken from these mines. No organisms active at pH 7.0-8.0 were detected in the water samples obtained from the Prince mine which had a pH value of 3.1. It is possible that methane oxidizing bacteria adapted to these conditions exist but no attempt was made to isolate them.

Although a large number of different colony types were present on the agar plates used during the isolation procedure, four types seemed to predominate. These were:

large pink colonies
micro pink colonies
medium yellow colonies
small colourless colonies

The large pink colonies were selected for further study since they appeared to be the fastest growing and produced the largest colonies on the agar plates. When samples of these organisms were grown in submerged culture the morphology changed slightly on re-plating. Subsequent cells grew as salmon coloured colonies on the agar. Microscopic examination showed this to be a mixed population containing two cell types, small Gram negative rods and cocci. The other colony types were not grown in submerged culture at this time.

The fact that the organisms were metabolically active in the presence of the coal mine waters was a good indicator that they would be active in the presence of coal. This was in fact, verified by the experiments described in Table 8 when it was shown that the organisms were actually more active when the flask in which they were cultured was filled with coal. There are two possible explanations for this phenomenon. Firstly, in nature, most micro-organisms prefer to grow on a solid support rather than to be suspended in a free floating state. This can be most dramatically illustrated in a study (22)on the microbial population of a river system. There it was shown that approximately 95% of the organisms were growing on suspended particles and only 5% were growing unsupported. Secondly, in the case of the flasks with the coal, the micro-organisms were growing in the thin film of water surrounding the coal and thus had better access to oxygen in the air. Shaking the flasks also causes an increase in activity since the bacteria grow adhering to the sides of the flasks and are wet with a thin film of water.

The results of static experiments are illustrated in Figures 2-6. Figure 2 shows that the rate of methane oxidation is relatively fast since a concentration of 12% could be oxidized in a 24 hour period. This was confirmed by the experiment shown in Figure 3 where the organisms were sufficiently active that no methane was detectable in flasks containing fresh coal. Flasks with no organisms added had levels of 7% methane within a few days.

Another factor which must be considered in the actual use of these organisms is the longevity of the microbial activity. The experiments shown in Figures 4-6 show that in the presence of coals containing low

levels of sulphur the activity was very long lasting. The organisms had lost only 40% of their activity after a period of 100 days. The longevity of the organisms was decreased if less cells were added (Figure 6) or if the Bushnell Haas medium used to wet the coal was replaced with distilled water (Figure 5).

The only parameter which appeared to have a negative effect on the microbial activity was the presence of high levels of sulphur in the coal since the bacterial activity remained high in the presence of low sulphur Western Canadian coals but was rapidly lost in the presence of a high sulphur Cape Breton coal (Prince: 5% sulphur). Very little activity was detected after 7 days with this coal. Although no experiments were carried out to test the hypothesis it is thought that sulphur oxidizing bacteria present in the samples converted the pyrite to sulphuric acid thus creating conditions i.e. low pH, unfavourable for the activity of the organisms. This hypothesis was supported by the finding of low pH values in this sample.

The results obtained from the dynamic test rig showed that the micro-organisms could consistently lower the methane concentration of a gas containing methane and air mixtures. This model system was equivalent to the use of the micro-organisms in underground coal mines where they could be sprayed on the walls or suspended in standing water along the floor of the abandoned mine workings.

Thus the work described in this report shows that the methane oxidizing bacteria isolated from a mine in S.E. British Columbia and grown under laboratory conditions can oxidize methane when in contact with coal. If scale up experiments are successful they could be used in a number of mining situations. Such situations would include:

coal storage silos
ship holds
in underground mines

In addition they could be used in other non-coal mining situations where methane build up could be a problem e.g. anaerobic waste treatment plants, sewage pipes and other underground mines where methane is present so long as the conditions found in these areas are similar in pH, temperature and ionic contaminants as the mine waters from the Western Canadian coal mines.

CONCLUSIONS

1. Methane oxidizing bacteria were found in all water samples from the mines in Western Canada where there was low levels of sulphur in the coal.

2. These organisms were easy to grow on simple media under laboratory conditions.

3. They were active in the presence of low sulphur Western Canadian coals.

4. They were inhibited when used in the presence of a high sulphur Cape Breton coal.

5. Their metabolic rates were such that it would appear that their use is justified on a larger scale.

6. They were active for long periods when added to low sulphur coal.

7. A dynamic test rig showed that they could be used underground in abandoned mine workings where there is either methane desorbing from the walls or there is a flow of methane through the mine tunnels.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. The physiological characteristics of the culture obtained from the Balmer South should be studied in more detail. In particular, the relationships between the bacterial activity and changes in pH and temperature should be studied in more detail.

2. The experiments on the model silo should be scaled up so that 2-5Kg of coal are treated at one time.

3. The other bacterial strains identified in the present study should be isolated, grown in larger quantities and characterized. A comparison of all the strains should be made to identify the strain with the most suitable properties for use in the different mining situations.

4. A simple bioreactor should be designed, built and tested so that an approximate economic assessment on the use of the methane oxidizing bacteria can be made.

REFERENCES

1. Suarez, J. and Chakravorty, R.N. "Methane Control in Coal Mines - a Review". ERP/MRL 76-108 (TR); July 1976.

2. Abetakis, M.G. "Methane Control in U.S. Coal Mines - An Overview" Symposium of the Bureau of Mines, Technology Rtansfer Seminar; pp. 9-17 1973.

3. "Firedamp Drainage" Published by the Coal Directorate of the Commision of the European Communities, Berlag Gluckuf, Essen, 1980.

4. Ivanov, M.V., A.M. Zyakum, V.A. Bondar, P.S. Litvinov, A.P. Petukh, V.I. Myaken'kii and B.I. Myagkii 1981. "Use of the Results of Analysis of the Isotopic Composition of Methane and Carbon Dioxide as an Indicator of the Microbiological Oxidation of Methane in Coal Mines". Doklady Akademii Nauk SSSR. 257(6): 1470-1473.

5. Abramov, R.A., Yu.R. Malashenko, V.I. Myaken'kii, I.K. Kurdish, G.A. Shevelov, P.S. Litvinov, and N.V. Voloshia 1977. "Vital Activity of Methane Oxidizing Bacteria after Filtration Through Coal Pourous Structures". Mikrobiologicheni; Jhurnal (Kiev) 39 (3) : 290-293.

6. Thakur, D.N. et al. "Laboratory and In-situ Investigations into Microbial Degassification of Coal Seams". Trans of the Mining, Metallurgical Institute of India; 79:67-92; 1983.

7. "Biological Control of Methane in Coal Mines - A Feasibility Study". CANMET Contract Report - Prepared by Gemini Biochenical Research Ltd., Calgary.

8. Kaserer, H. 1905. "Ueber Die Oxydation des Wasserstoffes and des Methanedurch Nicroorganismes". Zentbl. Bakt. Parasitkde. Abt. II. 15: 573.

9 Sohngen, N.L. 1906. "Ueber Bakterian Welche Methan als Koklerstoffrakrung and Energiequelle Gebrauchen". Zentbl. Bakt. Parasitkde. Abt. II pp. 15:513.

10 Wolfe, R.S. and I.J. Higgins. 1979."Microbiol Biochemistry of Methane - a Study of Contrasts". Int. Rev. Biochem. Microb. Biochem. 21: pp. 267-353.

11. Wolfe, H.J. and R.S. Hanson. 1979. "Isolation and Characterization of of Methane Utilizing Yeasts". J. Gen. Microbiol. 114: pp. 177-179.

12. Smirnova, Z.S. and L.v. Golubeva. 1969. "Microorganisms Which Oxidize Gaseous Hydrocarbons". Appl. Biochem. Microbiol. 5: pp. 499-505

13. Malashenko, Yu. R. and V.A. Romanouskaya. 1978. "Theoretical and Applied Aspects of the Study of Methane Oxidizing Bacteria". Mikrobiologichnii Zhurnal (Kiev). 40:(5): pp. 105-110.

14. Ivanov, M.V., A.I. Nesterov, B.B. Namsaraev, V.F. Gelchenko and A.V. Nazarenko. 1978. "Distribution and Geochemical Activity of Methanotrophic Bacteria in Coal Mine Waters". Mikrobiologiya. 47(3) : 489-494.

15. Goldlewska - Lipowa, W.A. 1979. "Control of Gas Hazards in Coal Mines with Bacteria". Prezeglad Gornicz 35 (6): 257-261.

16. Mueller,J.C. 1969a. "Fermentation of Natural Gas with Acyclone Column Fermenter". Can. J. Microbiol. 15: 1047-1050

17. Mueller,J.C. 1969b. "Preferential Utilization of the Methane Component of Natural Gas by a Mixed culture of Bacteria". Microbiol. 15: 1114-1116.

18. Roth-wood J. !983. Personnal communication. Gemini Biochemical Research Ltd. Calgary, Alberta.

19. Foster, J.W. and R.H. Davis. 1966. "A Methane Dependent Coccus, with notes on Classification, and Nomenclature of Obligate, Methane Utilizing Bacteria". . Bacteriol. 91: 1924-1931.

20. Leadbetter, E.R. and J.W. Foster. 1958. "Studies on some Methane Utilizing Bacteria". Arch. fur Mikrobiol. 30: pp. 91-118.

21. Wittenberg, R., Colby, J. and Reed, H.L. "Enrichment, Isolation and some Properties of Methane Utilizing Bacteria". J. Gem Microbiol. 114, 215-218, 1970.

22. Geesey, G.G. and Costerton, J.W. "Microbiology of a Northern River: Bacterial Distribution and Relationship to Suspended Sediment and Organic Carbon". Can. J. Microbiol. 25, 1058-1062; 1979.

APPENDIX A.

Experimental Details

Gases were analyzed on a Perkin Elmer Sigma 3B gas Chromatograph using the following conditions:

column	Supelco Carbosieve SII, 10 X 1/8 S.S.
oven temperature	65°C for oxygen, 225°C for methane and Carbon dioxide
injector temperature	250°C
detector temperature	250°C
carrier gas	helium @ 30ml/min
detector	hot wire
sample loop	0.1ml

pH measurement was made using a combination electrode and a Fisher Accumet pH meter (model 425 Digital).

Cations were measured using plasma emission spectroscopy by Metro Tech Systems Ltd. Anions were measured using ionic chromatography by Chemex Labs (Alberta) Ltd.

POOR

QUALITY

ORIGINAL